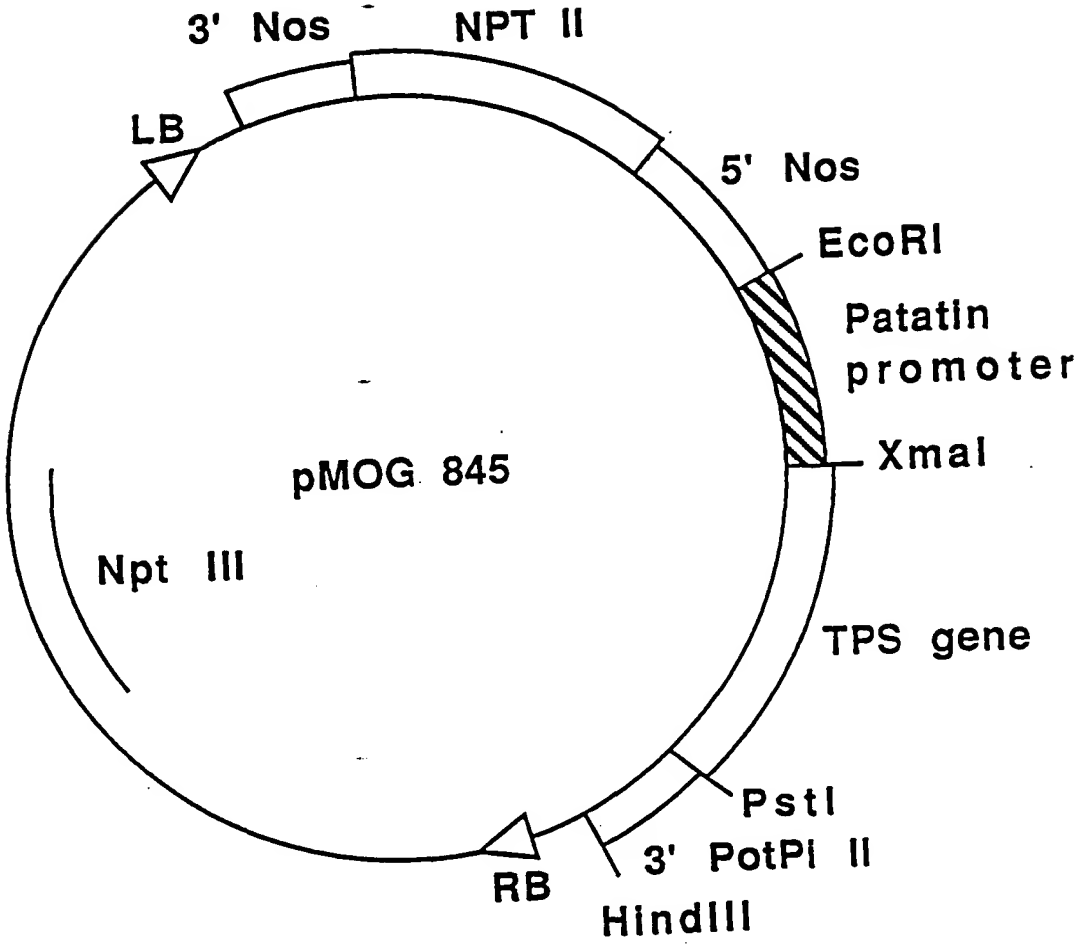

Fig. 1

EP 0 549 080 A2

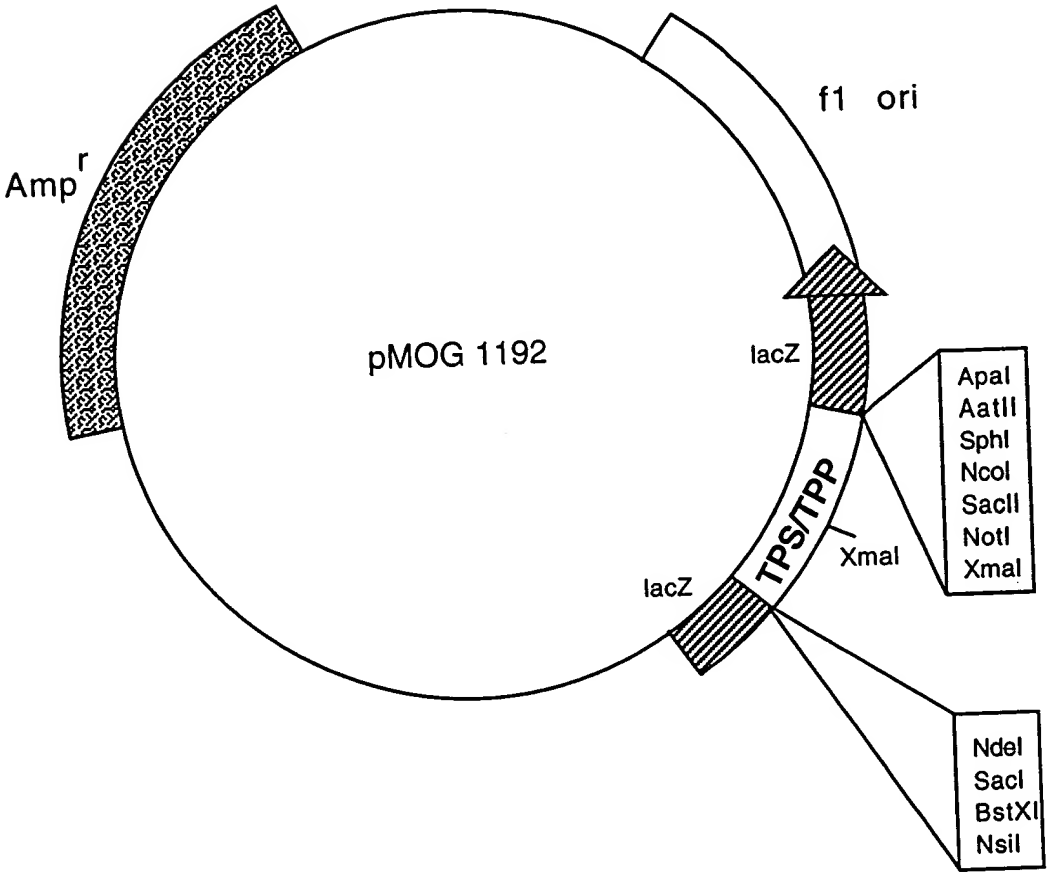
Amp r
f1 ori
pMOG 1192
lacZ
TPS/TPP
lacZ
ApaI
AatII
SphI
NcoI
SacII
NotI
XmaI
XmaI
NdeI
SacI
BstXI
NsiI

Fig. 2

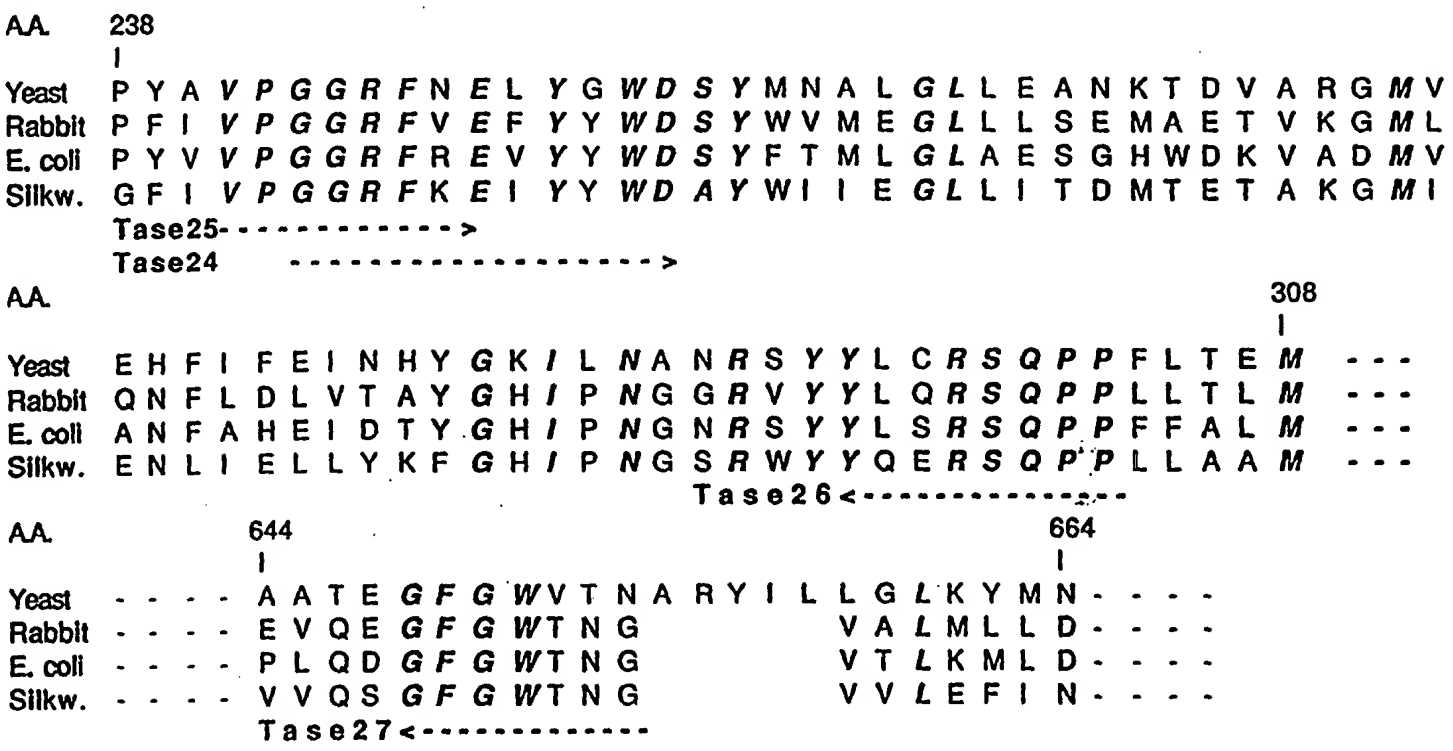
AA. 238
Yeast P Y A V P G G R F N E L Y G W D S Y M N A L G L L E A N K T D V A R G M V
Rabbit P F I V P G G R F V E F Y Y W D S Y W V M E G L L L S E M A E T V K G M L
E. coli P Y V V P G G R F R E V Y Y W D S Y F T M L G L A E S G H W D K V A D M V
Silkw. G F I V P G G R F K E I Y Y W D A Y W I I E G L L I T D M T E T A K G M I
Tase25->
Tase24 ->
AA. 308
Yeast E H F I F E I N H Y G K I L N A N R S Y Y L C R S Q P P F L T E M - - -
Rabbit Q N F L D L V T A Y G H I P N G G R V Y Y L Q R S Q P P L L T L M - - -
E. coli A N F A H E I D T Y G H I P N G N R S Y Y L S R S Q P P F F A L M - - -
Silkw. E N L I E L L Y K F G H I P N G S R W Y Y Q E R S Q P P L L A A M - - -
Tase26<-
AA. 644 664
Yeast - - - - A A T E G F G W V T N A R Y I L L G L K Y M N - - - -
Rabbit - - - - E V Q E G F G W T N G V A L M L L D - - - -
E. coli - - - - P L Q D G F G W T N G V T L K M L D - - - -
Silkw. - - - - V V Q S G F G W T N G V V L E F I N - - - -
Tase27<-

Fig. 3

Comparison of trehalase sequences derived from different species.

```
           1                                                  50
Ecoli2treh ..........  .........  ..MLNQKIQN PNPDELMIEV DLCYELDPYE
Ecolitreha ..........  .........  ...MKSPAPS RPQKMALIPA CIFLCFAALS
Bommotreha ..........  .........  ..........  ......MRLF LLLVGLTTV.
Tenmotreha ..........  .........  ..........  ......MIPF LLMVAFADTV
Rabbitreha ..........  .........  ..........  MPGSTWELHL LLLLGLG...
Potatotreha ..........  .........  ..........  ..MGKAIIFM IFTMSMNMIK
Yeasttreha MSVFDNVSPF KKTGFGKLQQ TRRGSEDDTY SSSQGNRRFF IEDVDKTLNE

           51                                                 100
Ecoli2treh LKLDEMIEAE PEPEMIEGLP ASDALTPADR YL.........  .....ELFEH
Ecolitreha VQAEETPVTP QPPDILLG..  ..........  ..........  .....PLFND
Bommotreha ..IADDLPPT CIRPVY....  .........C NS.........  .....TLLHY
Tenmotreha LQVSAQSQPS CDSKVY....  .........C QG.........  .....KLLHV
Rabbitreha LGSEQALPPP CESQIY....  .........C HG.........  .....ELLHQ
Potatotreha AETCKSIDKG PVIPTT....  ..........P LV.........  .....IFLEK
Yeasttreha LLAAEDTDKN YQITIEDTGP KVLKVGTANS YGYKHINIRG TYMLSNLLQE

           101                                                150
Ecoli2treh VQSAKIFP..  ....DSKTFP DCAPKMDPLD ILIRYRKVRR HRDF.....D
Ecolitreha VQNAKLFP..  ....DQKTFA DAVPNSDPLM ILADYRMQQN QSGF.....D
Bommotreha VQMARLYP..  ....DSKTFV DFQMRKDENA TLSAFQELLD RTNHNPTKED
Tenmotreha VEMSRIFN..  ....DSKTFV ELKMINDEQT TLENFDNFLR DTNHKRTRAD
Rabbitreha VQMARLYP..  ....DDKQFV DMPLSTAPDQ VLQSFAELAA TYNNTVPREQ
Potatotreha VQEAALQTYG HKGFDAKLFV DMSLRESLSE TVEAFNKLPR VVNGSISKSD
Yeasttreha LTIAKSFGRH QIFLDEARIN ENPVNRLSRL INTQF.....  .WNSLTRRVD

           151                                                200
Ecoli2treh LRKFVENHFW L.........  .........P EVYSSEYVSD PQN.SLKEHI
Ecolitreha LRHFVNVNFT L.........  .........P KE.GEKYVPP EGQ.SLREHI
Bommotreha LQEFVVDFFD E.TSELEEWK PDDHK..ENP P.FLAKIRDQ GFR.EFAKAL
Tenmotreha LMKFVSDNFK Q.ENEFESWT PTDFT..DNP T.LLSRIEDK TIR.QFAQDL
Rabbitreha LEKFVQEHFQ AVGQELESWT PGDWK..ESP Q.FLQKISDP KLR.AWAEQL
Potatotreha LDGFIGSYLS SPDKDLVYVE PMDFV..AEP EGFLPKVKNS EVR.AWALEV
Yeasttreha LNNVGEIAKD TKIDTPGAKN PRIYVPYDCP EQYEFYVQAS QMHPSLKLEV

           201                                                250
Ecoli2treh DQLWPVLTRE PQDHI....P WSSLLALPQ. ..........  ..SYIVPGGR
Ecolitreha DGLWPVLTRS TENTE....K WDSLLPLPE. ..........  ..PYVVPGGR
Bommotreha NDIWPTLARR VKPSVLEKPE QSSLVPMTH. ..........  ..GFIVPGGR
Tenmotreha VKIWPTLARK VKKEVLDYPE HYSLLPVDN. ..........  ..GFIIPGGR
Rabbitreha HLLWKKLGKK IKPEVLSQPE RFSLIYSQH. ..........  ..PFIVPGGR
Potatotreha HSLWKNLSRK VADHVLEKPE LYTLLPLKN. ..........  ..PVIIPGSR
Yeasttreha EYLPKKITAE YVKSVNDTPG LLALAMEEHF NPSTGEKTLI GYPYAVPGGR

           251                                                300
Ecoli2treh FSETYYWDSY FTMLGLAESG REDLLKCMAD NFAWMIENYG HIPNGNRTYY
Ecolitreha FREVYYWDSY FTMLGLAESG HWDKVADMVA NFAHEIDTYG HIPNGNRSYY
Bommotreha FKEIYYWDAY WIIEGLLITD MTETAKGMIE NLIELLYKFG HIPNGSRWYY
Tenmotreha FTEFYYWDSY WIVEGLLLSD MHETVRGMLD NFLSIVEKYG FIPNGARVFY
Rabbitreha FVEFYYWDSY WVMEGLLLSE MAETVKGMLQ NFLDLVTAYG HIPNGGRVYY
Potatotreha FKEVYYWDSY WVIRGLLASK MYETAKGIVT NLVSLIDQFG YVLNGARAYY
Yeasttreha FNELYGWDSY MMALGLLEAN KTDVARGMVE HFIFEINHYG KILNANRSYY
```

Fig. 4-1

09/980,050 - 05/29/02

```
            301                                                  350
Ecoli2treh  LSRSQPPVFA LMVELFEEDG VR......GA ..RRYLDHLK MEYAFWMDGA
Ecolitreha  LSRSQPPFFA LMVELLAQHE GD......AA .LKQYLPQMQ KEYAYWMDGV
Bommotreha  QERSQPPLLA AMIKLYYEKT KD......IE FIRKYISALE KELEYWLDT.
Tenmotreha  LNRSQPPLLT LMVSLYVSAT ND......ME WLAKNIRTID TELRFWLNN.
Rabbitreha  LQRSQPPLLT LMMDRYVAHT GD......LA FLRENIETLA LELDFWAEN.
Potatotreha SNRSQPPVLA TMIVDIFNQT GD......LN LVRRSLPALL KENHFWNSGI
Yeasttreha  LCRSQPPFLT EMALVVFKKL GGRSNPDAVD LLKRAFQASI KEYKTVWTAS

            351                                                  400
Ecoli2treh  ESLIPNQAYR HVVRMPDGSL LNRYWDDRDT PRDESWLEDV ETAK.HSG.R
Ecolitreha  ENLQAGQQEK RVVKLQDGTL LNRYWDDRDT PRPESWVEDI ATAKSNPN.R
Bommotreha  .HLIA.....  .FNKDDRVYT LLRYYIPSAG PRPESYYEDY ELAQKLDKNT
Tenmotreha  .RLVD.....  .VVKDGIVYK LAQYNSNSGS PRPESYYEDV TTASVFSDER
Rabbitreha  .RTIS.....  .VSSGGNSHT LNRYHVPYGG PRPESYSKDT ELAHTLPEG.
Potatotreha HKVTI.....  .QDAQGSNHS LSRYYAMWNK PRPESSTIDS ETASVLPNIC
Yeasttreha  PRL.......  .....DPETG LSRYHPNGLG IPPETESDHF DTV.LLPYAS

            401                                                  450
Ecoli2treh  PPNEVYRDLR AGAASGW...  .DYSSRWL..  ...RDTGRLA SIRTTQFIP.
Ecolitreha  PATEIYRDLR SAAASGW...  .DFSSRWM..  ...DNPQQLN TLRTTSIVP.
Bommotreha  DPNDIYADLK SAAESGW...  .DFSTRWFIS  ESGDNSGNLT NLNTKNVIP.
Tenmotreha  DKAELYMDLK SAAESGW...  .DFSSRWIVD  EYGGTRGNLS ALHTRRIIP.
Rabbitreha  SWETLWAELK AGAESGW...  .DFSSRWLVG  ..SPNPDSLG SIRTSKLVP.
Potatotreha EKRELYRELA SAAESGW...  .DFSSRWM..  ...SNGSDLT TTSTTSILP.
Yeasttreha  KHGVTLDEFK QLYNDGKIKE  PKLDEFFLHD  RGVRESGHDT TYRFEGVCAY

            451                                                  500
Ecoli2treh  ...IDLNAFL FKLESAIANI SALKGEKE..  ........TE ALFRQKASAR
Ecolitreha  ...VDLNSLM FKMEKILARA SKAAGDNA..  ........MA NQYETLANAR
Bommotreha  ...VDLNAIF AGALQITANF QAILKNPR..  ........RA AHWGYMAEQW
Tenmotreha  ...VDLNAFL CQAFQKLSEF YQTLGDYP..  ........NA TFWSKLVKIW
Rabbitreha  ...VDLNAFL CQAEELLSGF YSRLGNES..  ........QA TKYRNLRAQR
Potatotreha ...VDLNAFL LKMELDIAFL ANLVGESS..  ........TA SHFTEAAQNR
Yeasttreha  LATIDLNSLL YKYEIDIADF IKEFCDDKYE  DPLDHSITTS AMWKEMAKIR

            501                                                  550
Ecoli2treh  RDAVNRYLWD DENGIYRDYD WRREQL....  .........A LFSAAAIVPL
Ecolitreha  QKGIEKYLWN DQQGWYADYD LKSHKV....  .........RN QLTAAALFPL
Bommotreha  RSSIEQALWD EEDGVWHDYD ILNNK.....  ........PRR YFYTSNLAPL
Tenmotreha  QHSIEMVHYN RDDGIWYDWD NELSQ.....  ........HRR MFFPSNFAPL
Rabbitreha  IAALTALLWD EDKGAWFDYD LENQK.....  ........KNH EFYPSNLTPL
Potatotreha QKAINCIFWN AEMGQWLDYW LTNSDTSEDI  YKWEDLHQNK KSFASNFVPL
Yeasttreha  QEKITKYMWD DESGFFFDYN TKIKH.....  ........RTS YESATTFWAL

            551                                                  600
Ecoli2treh  YVGMANHEQA DRLANAVRSR LLT......P  GG...ILASE YETGEQWDKP
Ecolitreha  YVNAAAKDRA NKMATATKTH LLQ......P  GG...LNTTS VKSGQQWDAP
Bommotreha  WMNAVEKPFL AKHGARVLEY LHESQALEYP  GG...VPVSL VNSGEQWDFP
Tenmotreha  WSETFDSRNA EILGEMAAEY FITQNMMDYH  GG...IPTSL SHTGEQWDYP
Rabbitreha  WAGCFSDPAI A...DKALQY LQDSQILNHR  HG...IPTSL QNTGQQWDFP
Potatotreha WTE.ISCSDN NITTQKVVQS LMSSGLLQ.P  AG...IAMTL SNTGQQWDFP
Yeasttreha  WAGLATKEQA QKMVEKALPK LEMLGGLAAC  TERSRGPISI SRPIRQWDYP
```

08/759,460 - 05/29/97

Fig. 4-2

```
            601                                                  650
Ecoli2treh  NGWAPLQWMA IQGFKMYG.. .DDLLGDEIA RSWLKTVNQF YLE.QHKLIE
Ecolitreha  NGWAPLQWVA TEGLQNYG.. .QKEVAMDIS WHFLTNVQHT YDR.EKKLVE
Bommotreha  NAWPPEVSIV VTAIQNIGSE ESSKLAKELA QVWVRACKSG FTE.KKQMFE
Tenmotreha  NAWPPMQSII VMGLDKSGSY RAKQLARELA RRWVKANLIG FRQ.TGEMFE
Rabbitreha  NAWAPLQDLV IRGLAKSPSA RTQEVAFQLA QNWIRTNFDV YSQ.RSAMYE
Potatotreha NGWPPLQHII IEGLLRSGLE EARTLAKDIA IRWLRTNYVT YKK.TGAMYE
Yeasttreha  FGWAPHQILA WEGLRSYGYL ...TVTNRLA YRWLFMMTKA FVDYNGIVVE

            651                                                  700
Ecoli2treh  KYHIADGVPR EGGGGEYPLQ .........D GFGWTNG... VVRRLIGLYG
Ecolitreha  KYDVSTTGT. GGGGGEYPLQ .........D GFGWTNG... VTLKMLDLIC
Bommotreha  KYDALNAGKY .GGGGEYTVQ .........D GFGWSNG... VVLEFLDRYG
Tenmotreha  KYNVEVPGQN .GGGGEYVVQ .........S GFGWTNG... VVLEFINQFF
Rabbitreha  KYDISNA.QP .GGGGEYEVQ .........E GFGWTNG... VALMLLDRYG
Potatotreha KYDVTKCGAY .GGGGEYMSQ .........T GFGWSNG... VVLALLEEFG
Yeasttreha  KYDVTRGTDP HRVEAEYGNQ GADFKGAATE GFGWVNARYI LGLKYMNSYE

            701                                  732
Ecoli2treh  EP..... ..... ..... ..
Ecolitreha  PKEQPCDNVP ATRPTVKSAT TQPSTKEAQP TP
Bommotreha  AVLTSVDSVD ASANNGQSNE ESETDSKEK. ..
Tenmotreha  TT..... ..... ..... ..
Rabbitreha  DRLSSGTQLA LLEPHCLAAA LLLSFLTR.. ..
Potatotreha WPEDLKIDC. ..... ..... ..
Yeasttreha  RREIGACIPP ISFFSSLRPQ ERNLYGL... ..
```

09/701,050 - 09/29/2000

Fig. 4-3

08279460 "052797

Trehalase activity in tobacco leaves transgenic for a potato trehalase cDNA

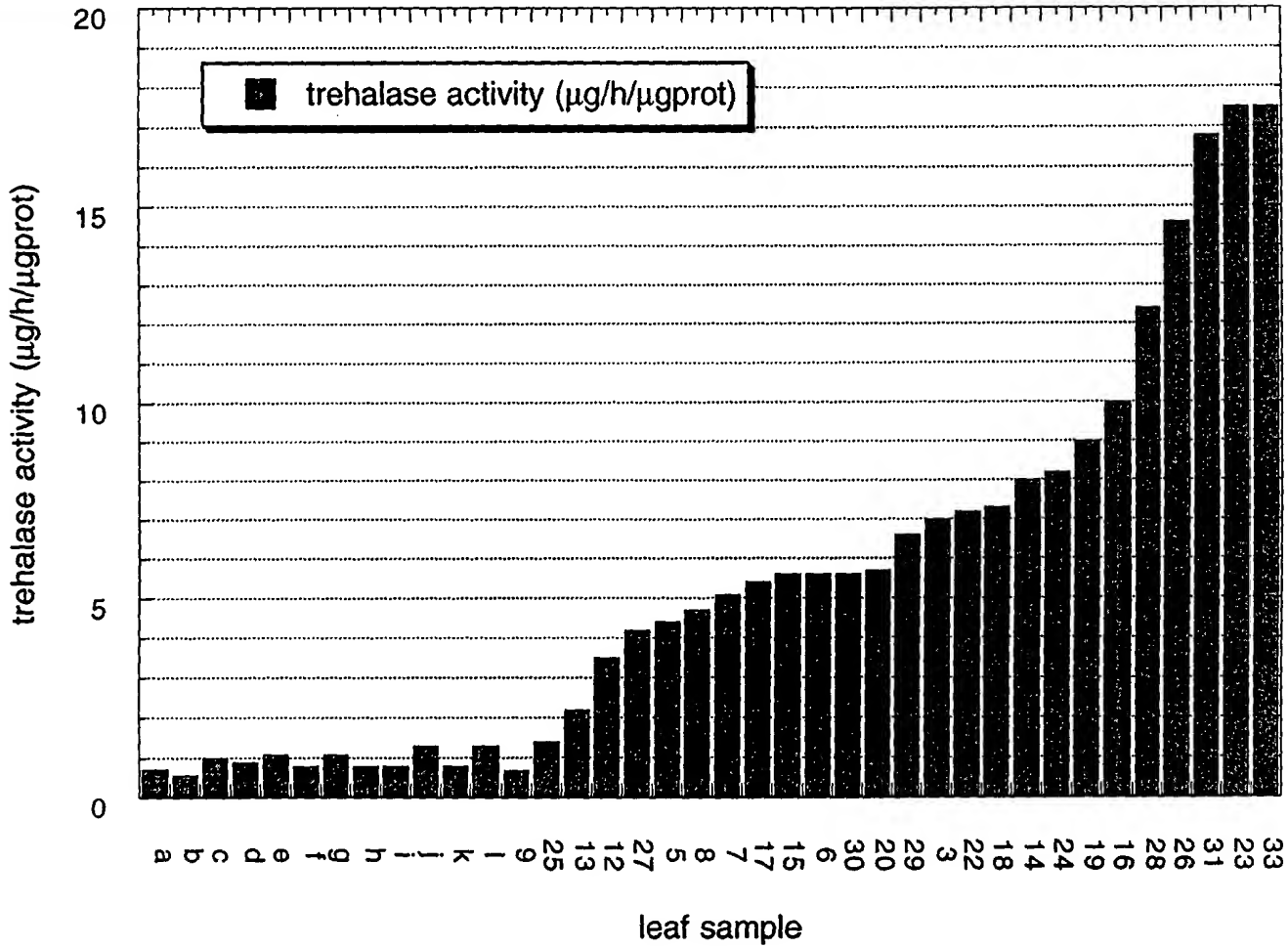

Fig. 5

Trehalose accumulation in microtubers

trehalose
mg.g^{-1} fresh weight

pMOG1027 (845-11)		pMOG1027 (845-22)		pMOG1027 (845-28)	
9a	0.08	7a	0.02	3a	0.04
9b	0.21	7b	0.01	3b	0.15
11a	0.01	9a	0.02	15a	0.4
11b	0	9b	0.02	15b	ND
...		12a	0.09	18a	0
...		12b	0	18b	0.2
...		15a	0	26a	0.12
...		15b	0.01	26b	0.22
...		32a	0.01	27a	0.02
...		32b	0	27b	0.01
...		34a	0.01	31a	0.2
...		34b	0.01	31b	0.1
...		36a	0.01	34a	0.48
...		36b	0	34b	0.75
...				38a	0.08
				38b	0.12
N= 25		N= 30		N= 29	

Fig. 6